PATENT COOPERATION TREA

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 17838E	FOR FURTHER ACTION See Form PCT/IPEA/416	
International application No. PCT/FI 2003/000596	International filing date *(day/month/year)* 07.08.2003	Priority date *(day/month/year)* 07.08.2002
International Patent Classification (IPC) or national classification and IPC G01N 33/58, G01N 15/14		
Applicant Cyflo Oy et al		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 5 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *(sent to the applicant and to the International Bureau)* a total of 6 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s))
      _____, containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☒ Box No. II Priority
   - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☐ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
10.02.2004	02.11.2004
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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

national application No.
PCT/FI 2003/000596

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This report is based on a translation from the original language into the following language _____, which is the language of a translation furnished for the purposes of:

☐ international search (under Rules 12.3 and 23.1(b))
☐ publication of the international application (under Rule 12.4)
☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report):*

☐ the international application as originally filed/furnished

☒ the description:

pages 1-38 as originally filed/furnished
pages* _____ received by this Authority on _____
pages* _____ received by this Authority on _____

☒ the claims:

pages _____ as originally filed/furnished
pages* _____ as amended (together with any statement) under Article 19
pages* 39-44 received by this Authority on 16-06-2004
pages* _____ received by this Authority on _____

☒ the drawings:

pages 1-5 as originally filed/furnished
pages* _____ received by this Authority on _____
pages* _____ received by this Authority on _____

☐ a sequence listing and/or any related table(s) – see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, sheets/figs _____
☐ the sequence listing *(specify)*: _____
☐ any table(s) related to the sequence listing *(specify)*: _____

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, sheets/figs _____
☐ the sequence listing *(specify)*: _____
☐ any table(s) related to the sequence listing *(specify)*: _____

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/FI 2003/000596

Box No. II Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

   ☐ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

The priority is considered valid. Consequently, the documents

Lionel Rigottier-Gois et al, Fluorescent hybridisation combined with flow cytometry and hybridisation of total RNA to analyse the composition of microbial communities in human faeces using 16S rRNA probes, FEMS Microbiology Ecology, Vol 43, page 237-246, 2003

Erwin G. Zoetendal et al, Quantification of uncultured ruminococcus obeum-like bacteria in human fecal samples by fluorescent in situ hybridization and flow cytometry using 16S rRNA-targeted probes, Applied and environmental microbiology, vol 68, No 9, page 4225-4232, 2002

are not considered in this examination report.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

national application No.
PCT/FI 2003/000596

Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-34	YES
	Claims		NO
Inventive step (IS)	Claims	1-34	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-34	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The most relevant documents cited in the International Search Report are:

D1: Wallner, G et al, Flow cytometric analysis of activated sludge with rRNA-Targeted Probes, Applied and Environmental Microbiology, Volume 61, No 5, page 1859- 1866, May 1995

D2: Langworthy, D E et al, Flow cytometric methods for the study of bacteria and protozoan parasites in environmental matrices, BIOSIS accession no. PREV200200616964

Document D1 relates to flow cytometric analysis of activated sludge. The target bacterial population is hybridized with one probe having one fluorochrome. To distinguish particles containing DNA from particles not containing DNA the hybridized sample is stained with a DNA colour that cannot be excited with the same laser as the fluorochrome of the probe. Therefore two argon lasers are used.

The objective is to achieve proportional measurement of the target bacterial population in relation to the rest of the bacteria contained in the sample.

Document D1 is considered to represent the closest prior art.

The invention according to claim 1 differs from D1 in that the difference in intensities of the mean flourescences of the fluorescent agents is at least about double on a logarithmic scale.

.../...

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

Due to these features, it is possible to separate 1) target micro organisms 2) other micro organisms and 3) other particles and background/noise of a sample in one analysis and to present said different groups in one graph.

The cited prior art does not give any indication that would lead a person skilled in the art to the choice of a method with difference in fluorescence intensities. Therefore, the claimed invention is not obvious to a person skilled in the art.

Accordingly, the invention defined in claims 1-34 is novel and is considered to involve an inventive step. The invention is industrially applicable.

CLAIMS

1. A method for identifying one or more micro-organism and/or micro-organism species, and for measuring the portion of at least one micro-organism and/or micro-organism species from a sample, characterised in that

a) binding to a structure individualising at least one micro-organism species or group and enabling identification a first fluorescent agent that absorbs light in a first wavelength area,
b) binding to a structure characteristic of all micro organisms a second fluorescent agent that absorbs light in a second wavelength area,
c) subjecting the sample to flow,
d) exciting the aforementioned first fluorescent agent in the aforementioned flow with a monochromatic light disposed in the first wavelength area,
e) exciting the aforementioned second fluorescent agent in the aforementioned flow with a monochromatic light disposed in the second wavelength area,
f) identifying the target micro-organism by analysing the fluorescence of the fluorescent agents bound to the particles of the sample,

and in that the fluorescent agents and the wavelength areas of the monochromatic light are chosen in such a manner that the difference in intensities of the mean fluorescences of the fluorescent agents is at least about double on a logarithmic scale.

2. The method according to claim 1, characterised in that the method further comprises a step at which the portion(s) of the identified target micro-organism(s) is/are calculated from the total amount of sample.

3. The method according to claim 1 or 2, characterised in that a measurable difference in intensities between the fluorescences of the fluorescent agents is achieved in the first wavelength area.

4. The method according to any one of claims 1-3, characterised in that the sample is introduced into a flow cytometer.

5. The method according to any one of claims 1-4, characterised in that a first fluorescent agent is attached to the probes that are bound to the structure individualising at least one micro-organism species or group in the sample and enabling the identification.

6. The method according to any one of claims 1-5, characterised in that a structure individualising one micro-organism species or group and enabling the identification is a ribosomal RNA molecule.

7. The method according to any one of claims 1-6, characterised in that a structure characteristic of all micro-organisms is DNA.

8. The method according to any one of claims 1-7, characterised in that a threshold value is set for each micro-organism for each parameter specifically, and the micro-organisms are classified based on their threshold values.

9. The method according to any of claims 1-8, characterised in that the fluorescent agent is a fluorochrome.

10. The method according to any one of claims 1-9, characterised in that the micro-organism is a bacterium and/or a bacterial species.

11. The method according to claim 10, characterised in that the aforementioned ribosomal RNA molecules are chosen from a group consisting of 16S ribosomal RNA molecules and 23S ribosomal RNA molecules.

12. The method according to any one of claims 1-11, characterised in that the light scattering from the particles of the sample is detected.

13. The method according to any one of claims 1-12, characterised in that micro particles are further separated from the sample based on their scattering and/or fluorescence properties.

14. The method according to any one of claim 1-13, characterised in that the first wavelength area is 600-650 nm.

15. The method according to any one of claim 1-13, characterised in that the second wavelength area is 350-600 nm.

16. The method according to any one of claims 1-15, characterised in that the monochromatic lights disposed in the first and second wavelength area are formed by one light source.

17. The method according to any one of claims 1-15, characterised in that the monochromatic lights disposed in the aforementioned first and second wavelength area are formed by at least two light sources.

18. The method according to claim 17, characterised in that at least two of the aforementioned at least two light sources are disposed at a distance from each other, and in that in the method, signal delay equipment is used to delay the measuring signals being created by means of the first and optionally the subsequent light sources.

19. The method according to any one of claims 1-18, characterised in that the sample is a sample from a mammal's organism fluid.

20. The method according to claim 19, characterised in that the sample is a sample originating from a mammal's digestive system.

21. The method according to any one of claims 1-18, characterised in that the sample is a waste water sample.

22. A device for identifying one or more micro-organisms and/or micro-organism species and for measuring the portion of at least one micro-organism and/or micro-organism species from the sample, characterised in that the device comprises:

a) a flow chamber (5), into which a solution being analysed (6) containing the sample is introduced, in which to a structure individualising at least one micro-organism species or group and enabling the identification, a first fluorescent agent is bound that absorbs light in a first wavelength area, and in which to a structure characteristic of all micro-organisms, a second fluorescent agent is bound that absorbs light in a second wavelength area,
b) a light source (1, 3) for producing a monochromatic light at different wavelengths,

c) one or more detectors (14, 15, 16, 17) for measuring the signal forming the fluorescent agent for identifying the target micro-organism,

and in which device the fluorescent agents of the sample and the wavelength areas of the monochromatic light are chosen in such a manner that the difference in intensities between the mean fluorescences of the fluorescent agents is at least double on a logarithmic scale.

23. The device according to claim 22, c h a r a c t e r i s e d in that the device further comprises calculation means for calculating the portion(s) of the identified micro-organism(s) from the total amount of sample.

24. The device according to claim 22 or 23, c h a r a c t e r i s e d in that a measurable difference in intensities between the fluorescences of the fluorescent agents is achieved in the first wavelength area.

25. The device according to any one of claims 22-24, c h a r a c t e r i s e d in that the device is a flow cytometer.

26. The device according to any one of claims 22-25, c h a r a c t e r i s e d in that the detector (14, 15, 16, 17) is used to detect the light scattering from the particles in the sample.

27. The device according to any one of claims 22-26, c h a r a c t e r i s e d in that the device further comprises a feeding device for dosing a standard amount of sample.

28. The device according to any one of claims 22-27, c h a r a c t e r i s e d in that the light source (1, 3) includes at least two light sources for producing the

aforementioned monochromatic lights disposed in the first and second wavelength area.

29. The device according to claim 28, c h a r a c t e r - i s e d in that at least two of the aforementioned at least two light sources are disposed at a distance from each other, and in that the device further comprises signal delay equipment for delaying the measuring signals being created by means of the first and optionally the subsequent light sources.

30. The device according to any one of claims 22-29, c h a r a c t e r i s e d in that the aforementioned light source(s) (1, 3) is/are chosen from a group consisting of a diode laser of 635 nm and an argon ion laser of 488 nm.

31. The use of a method according to any one of claims 1-21 for identifying micro-organisms and for measuring their portions.

32. The use according to claim 31, c h a r a c t e r - i s e d in that the micro-organism is a probiotic bacterial strain.

33. The use of a device according to any one of claims 22-30 for identifying micro-organisms and for measuring their portions.

34. The use according to claim 33, c h a r a c t e r - i s e d in that the micro-organism is a probiotic bacterial strain.